Notes on the Design of Bioequivalence Study: Naloxone (Nasal)

Notes on the design of bioequivalence studies with products invited for submission to the WHO Prequalification Unit – Medicines Assessment Team (PQT/MED) are issued to aid manufacturers with the development of their product dossier. Deviations from the approach suggested below can be considered acceptable if justified by sound scientific evidence.

The current notes should be read and followed in line with the general guidelines of submission of documentation for WHO prequalification. In particular, please consult the "Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability" in: *Fifty-seventh Report of the WHO Expert Committee on Specifications for Pharmaceutical Preparations*. Geneva, World Health Organization, 2024. WHO Technical Report Series, No. 1052, Annex 8.

Below, additional specific guidance is provided on the invited nasal products containing naloxone.

Pharmacokinetics of naloxone

After nasal administration, median t_{max} was observed at 15 min, 20 min, 30 min, 45 min depending on the product concentration and number of actuations (i.e., one spray administration in one nostril or one spray administration in each nostril), with a shorter t_{max} observed with more concentrated solutions.

Following a single intranasal administration, the mean half-life ($t_{1/2}$) of naloxone in plasma ranged between 1.33 to 2.7 hours, which is slightly longer than the half-live observed after intravenous administration, e.g., 1.22 h (i.e., absorption-rate limited elimination).

Guidance for the design of bioequivalence studies

Taking into account the pharmacokinetic properties of naloxone, the following guidance with regard to the study design should be taken into account:

Design: A single-dose, crossover design is recommended.

Dose: The EoI includes formulations for intranasal use (2 mg/0.1ml, 3 mg/0.1 ml, 4 mg/0.1 ml) for which a waiver based on in vitro characterisation might also be possible when pharmaceutically equivalent products are aqueous solutions intended to be administered with essentially the same device, contain the same APIs in the same concentration, and contain the same excipients in similar concentrations.

When bioequivalence is shown in vivo, the dose should be administered as one spray in one nostril.

If bioequivalence of the 4 mg/spray strength is demonstrated, the additional strengths could be waived from the in vivo demonstration of bioequivalence be based on the in vitro tests described below as option for a full biowaiver. However, the drug in small particles/droplets and plume geometry tests may not be needed for the additional strengths provided they are manufactured without changing the actuator and metering valve or pump (other than diptube, due to different volumes of product or other factors) used in the 4 mg/spray strength. Recommendations for in vitro bioequivalence testing at various life stages are not relevant for these products, since they are a single-use configuration.

Fasted/fed: N/A

Subjects: Healthy adult subjects should be used. It is not necessary to include patients in the bioequivalence study.

Parent or metabolite data for assessment of bioequivalence: The parent drug is considered to best reflect the biopharmaceutical quality of the product. Therefore, bioequivalence should be based on the determination of naloxone.

Sample size: Information on naloxone nasal spray currently available to the PQT/MED indicates that the intra-subject variability is around 46% for C_{max} and 34% for AUC_{0-t}. These data will facilitate the calculation of sufficient sample size for the cross-over bioequivalence study.

Washout: Taking into account the elimination half-life of naloxone in healthy volunteers (2.7 hours), a washout period of 1 day is considered sufficient to prevent carry over.

Blood sampling: As naloxone is absorbed rapidly and has a short half-life, blood sampling should be intensive in the first minutes after administration to cover the peak of naloxone. Sufficient quantifiable samples should be taken to allow a comparison of exposure to naloxone within the initial 4 minutes, first 10, 20 and 30 minutes after administration, e.g., pre-dose sample and samples at 2, 4, 6, 8, 10, 15, 20, 25, 30, 35, 40, 45, 60 minutes and 1.5, 2, 3, 4, 6 and 8 hours after administration.

Analytical method: Information currently available to the PQT/MED indicates that it is possible to measure naloxone in human plasma using LC-MS/MS analytical methodology. The bioanalytical method should be sufficiently sensitive to detect concentrations that are 5% of the Cmax in most profiles of each formulation (test or comparator). See Guideline on bioanalytical method validation and study sample analysis. In: WHO Technical Report Series, No. 1060, Annex 6, or the ICH Harmonised Guideline M10 for more information on bioanalytical recommendations.

Statistical considerations: The data for naloxone should meet the following bioequivalence standards in a single dose cross-over design study:

- The 90% confidence interval of the relative mean AUC_{0-t} of the test to reference product should be within 80-125%.
- The 90% confidence interval of the relative mean C_{max} of the test to reference product should be within 80-125%.
- The 90% confidence interval of the relative mean early partial $AUC_{0-4\ minutes}$ of the test to reference product should be reported as supportive information to assess the equivalent onset of naloxone effect.
- The 90% confidence interval of the relative mean early partial $AUC_{0-10\ minutes}$ of the test to reference product should be reported as supportive information to assess the equivalent onset of naloxone effect.
- The 90% confidence interval of the relative mean early partial $AUC_{0-20\ minutes}$ of the test to reference product should be reported as supportive information to assess the equivalent onset of naloxone effect.
- The 90% confidence interval of the relative mean early partial $AUC_{0-30\ minutes}$ of the test to reference product should be reported as supportive information to assess the equivalent onset of naloxone effect.
- The 90% non-parametric confidence interval of t_{max} of the test and reference product expressed as difference in minutes should be reported as supportive information to assess the equivalent onset of naloxone effect.

Information currently available to PQT/MED suggests that the comparator product is a highly variable drug product for both AUC_{0-t} and C_{max}. Therefore, if the applicant suspects that the variability of C_{max} or AUC_{0-t} is high (CV>30%), the applicant may prefer to employ a full replicate, crossover study in order to widen the acceptance range of C_{max}

and/or AUC_{0-t}. For more information on replicate study designs and widening the acceptance limits of average bioequivalence based on the intra-subject variability of the comparator product, refer to Section 7.9.3 of Annex 8, TRS 1052 and PQT/MED guidance document "Application of reference-scaled criteria for AUC in bioequivalence studies conducted for submission to PQT/MED".

Waiver:

The waiver of the in vivo demonstration of bioequivalence could be justified if:

- The test product contains the same excipients (Q_1) in similar amounts (Q_2) within ±5% of those used in the comparator product.
- In vitro similarity in the following in vitro tests is shown between samples of at least three batches of the text and the comparator products with no fewer than 10 units from each batch based on average bioequivalence. Otherwise, follow the US FDA methodology.

  1. Single actuation content.

  2. Droplet size distribution by laser diffraction

  3. Drug in small particles/droplets

  4. Spray pattern

  5. Plume geometry

  The three batches of the test product should be manufactured from, at minimum, three different batches of the drug substance, three different batches of critical excipients, and three different batches of the device components (e.g., pump and actuator) proposed for the final device configuration of the commercial product. The test product should consist of the final device constituent part and final drug constituent formulation intended to be marketed.

Additional information:

Device:

The size and shape, the external critical design attributes, and external operating principles of the comparator product device should be mimicked including:

- Single-use, single-dose format
- No priming
- Metered or non-metered spray

If bioequivalence is shown in vitro, i.e., full biowaiver, each strength of the test should be compared with the in vitro test described above with the corresponding strength of the comparator.

User interface assessment:

Comparative analyses in a Human Factors Study should be submitted for the applied drug - device combination to assess the user interface and determine whether any differences in design for the user interface of the applied multisource (generic) product are acceptable and whether the applied multisource (generic) product can be

expected to have the same clinical efficacy and safety profiles as the comparator product when administered according to the dosing instructions. For additional information, refer to the most recent version of the FDA guidance for industry on *Comparative Analyses and Related Comparative Use Human Factors Studies for a Drug-Device Combination Product Submitted in an ANDA*.